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'Surface Transplantation' for Nerve Injury and Repair: The Quest for Minimally Invasive Cell Delivery

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Highlights

- Compression injury to the auditory nerve causes auditory spiral ganglion cell death and cochlear cell death. Subsequently, reactive astrocytes project numerous processes into the peripheral portion of the nerve (the astrocyte outgrowth).
- Donor cells transplanted on the surface of the astrocyte outgrowth can autonomously enter the nerve, migrate, and integrate into the host leading to functional restoration. In contrast, when donor cells are injected into the nerve, they die.
- Important structural and biochemical cues for axon regeneration embedded in the host can be harnessed by donor cells only when they are transplanted on the surface of the host, without disturbing astrogliosis/astrocyte scar.
- We hypothesize that surface transplantation may be applied not only for motor neuron diseases but also for Alzheimer's disease and Parkinson's disease in which the lesions lie deeper within the CNS.

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Title

"Surface Transplantation" for Nerve Injury and Repair: The Quest for Minimally-invasive Cell Delivery

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Key words: Astrocyte scar, Cell transplantation, Nerve injury, Neurodegenerative disorders, Reactive astrocyte, Surface transplantation

Short title: Surface transplantation for neurodegenerative disorders

Abstract

Cell transplantation is ambitious but arguably realistic for repair of the nervous system. Cell delivery is a major challenge for clinical translation, especially given the apparently inhibitory astrogliotic environment in degenerated tissue. However, astrogliotic tissue also contains endogenous structural and biochemical cues that can be harnessed for functional repair. Minimizing damage to these cues during cell delivery could thus be harnessed for enhancing cell integration. This is supported by work with an auditory

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astrocyte scar model, in which cells delivered onto the surface of the damaged nerve were more successfully integrated in the host than those injected into the tissue. We consider the application of this less invasive approach for nerve injury and its potential application to some neurodegenerative disorders.

Cell transplantation and the astrocyte scar

Replacement of lost neurons by cell transplantation has long been investigated as a possible treatment for spinal cord injury (SCI), and more recently also for neurodegenerative disorders (NDDs), including **Parkinson's disease** (PD) (see Glossary), **amyotrophic lateral sclerosis** (ALS), **Huntington's disease** (HD), **multiple sclerosis** (MS), **temporal lobe epilepsy** (TLE) and **Alzheimer's disease** (AD) [1-7]. There is recent, encouraging evidence for successful integration of transplanted, exogenous grafts into host tissue [1-4] and in some cases it is associated with evidence for measurable improvement of patients' signs and symptoms [5, 6]. Nevertheless, there are numerous hurdles to overcome for full clinical application, not least because astrogliosis, especially the astrocyte scar (the glial scar), is believed to generate mechanical and molecular barriers against regenerating axons [8, 9].

In this article we discuss a new and more positive view of the astrocyte scar and argue that its *surface* can play a pivotal role in the functional integration of donor cells. This is based on our experiments with an auditory astrocyte scar model (Box 1) in which donor cells (Box 2) placed on the surface of the nerve can enter the host tissue autonomously, migrate and differentiate to establish synaptic connections with peripheral and central targets and to restore measurable recovery of auditory function [10] (Figure 1) (Key Figure). We speculate that surface transplantation could be an effective way of exploiting the astrocyte scar in other kinds of nerve injury and even in some forms of NDDs.

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Intraparenchymal injection versus surface transplantation

The most reliable method of cell transplantation has intuitively been believed to be direct injection of cells into the host nervous tissue, although there is little comparative work examining efficacy of different delivery strategies [11, 12]. In contexts where an astrocytic scar is involved, it would be a major advantage, we believe, if one could overcome the inhibition of the astrocyte scar using a less invasive approach that causes minimal destruction of healthy nerve tissue and supports maximal functional integration of donor cells. In this article we revisit critical aspects of intraparenchymal injection in the light of an alternative approach based upon surface transplantation.

Mechanical damage to nervous tissue during intraparenchymal injection.

A study by Wiesmann et al. demonstrated that even a small volume of Ringer's solution intraneurally injected into pig brachial plexus nerves can induce local inflammation and myelin damage within the nerve [13]. In another experimental study in which human adipose-derived stem cells (hASCs) were epineurally or intraneurally injected into injured sciatic nerves of rats, improvement in mechanical allodynia was higher, and the results of the paw withdrawal test were better in the former compared to the latter group [11]. The authors concluded that intraneural injection is invasive and negates the beneficial effect of hASCs. Also in the CNS, it is very likely that devices used for intraparenchymal injection, such as a syringe needle, mechanically injure CNS tissue. By itself, this can cause trauma that leads to activation of immuno-inflammatory cells and astrocytes [14-16]. Infusion pressure during cell or viral vector injection and the resulting cell or vector mass may damage residual functional neurons and their associated vascular networks [11] (see below), which can also reactivate astrocyte scar formation. Cells and viral vectors that cross the physiological border may cause unexpected and detrimental electrical over-activity not only of neurons around the lesion but also of distant

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neuronal networks. In a study of ALS patients, donor cells were injected into up to 20 locations in the spinal cord via a needle penetrating the dorsal surface to reach the ventral horn. Serious complications, such as bilateral lower leg weakness and continued central pain syndrome were observed in some cases [17]. The outcome might have been more favorable with a less invasive method.

Adaptation of donor cells to the host environment?

Donor cells delivered intraparenchymally are placed abruptly into an alien, pathological host environment [7, 12] and are not supported by an immediate blood supply [15], leading to large scale donor cell death [18]. By contrast, in surface transplantation, donor cells are placed in cerebrospinal fluid (CSF) – known to be a major nourishing source for the central nervous system (CNS) [19] – before they establish a link to the blood supply. Somewhat surprisingly, pro-regenerative molecules, including brain-derived neurotrophic factor (BDNF) and insulin-like growth factor 2 (IGF-2), are produced in the choroid plexus in the ventricle and released into the CSF. Thus it has been hypothesized that the CSF plays a crucial role in protecting the brain parenchyma against detrimental changes in the aged brain, in PD and in MS [20, 21]. In our experiments with the auditory system, multiple layers of donor cells were observed on the surface of the auditory nerve for as long as 3 months after transplantation (Fig. 3C and D in ref. #10). The survival of these cells is likely to have depended initially on CSF nutrients and later also on nutrients from new host blood vessels. Following intraparenchymal injection the majority of donor cells die [10], possibly through lack of immediate support from the CSF and then the blood supply. BDNF was highly expressed in our astrogliotic auditory nerve model [10] and it might aid survival of donor cells both inside the auditory nerve and outside in the CSF. It also seems reasonable to assume that less invasive cell delivery techniques minimize

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disruption of existing blood vessels and facilitate more efficient vascularization of the donor cell mass.

What is the optimum number of donor cells?

In intraparenchymal injection, estimating the optimum number of donor cells for delivery is difficult. The death rate is often very high, surpassing 90% in some studies [18]. Uncontrollable growth of a graft within the brain could lead to devastating consequences such as brain herniation and death [22], so it is desirable to develop a method of delivery that matches the number of donor cells to the capacity of the host environment. Following surface delivery to the auditory nerve we observed donor cells apparently entering the tissue some 2 to 3 months after transplantation (Figs. 3F/G, 2 months post-transplantation; and 3E, 3 months post-transplantation in ref. #10), suggesting that cell entry may continue over a long period. During development, the number of oligodendrocyte precursor cells (OPCs) is proportional to the supply of platelet-derived growth factor (PDGF), the main mitogen for OPC, and the final number of cells appears to be regulated primarily by PDGF levels [23]. If this principle applies to the migration of donor cells following surface transplantation, then the number of donor cells applied would be less critical as it might be functionally related to the host environment. Further studies are warranted to investigate potential interactions between donor cells and the host environment to enable long-lasting, regulated cell entry in surface transplantation.

Mobilization of transplanted cells to the lesion

Experimental evidence for signaling from damaged tissue to donor cells came from a study on a rat model of stroke, following transplantation with conditionally immortal human neural stem cell line (MHP36 cells) [24] similar to the N33 cells used in our study [10] (see Box 2). The donor cells migrated from the injection site to the infarcted lesion and significant improvements

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in both sensorimotor function and gross motor asymmetry were observed at 6–12 weeks post-grafting [24]. In addition to stroke, similar homing phenomena have been reported in various diseases including SCI and inflammatory diseases [25-27]. It seems then that donor cells transplanted on the surface of the CNS could potentially be mobilized to the lesion site once they crossed the pia mater and entered the CNS, although the specific mechanisms mobilizing transplanted cells to the damaged tissue remain to be clarified.

Donor cells can enter the CNS from its surface

CSF circulates into the parenchyma of the brain and spinal cord along perivascular spaces [28, 29], and in some studies, intrathecally injected cells have been shown to follow the same route [30, 31]. In a mouse model of MS, adult neural stem cells injected into the subarachnoid space (SAS) entered into demyelinating areas of the CNS and promoted remyelination with functional recovery, indicating that donor cells can pass through the brain surface under inflammatory conditions [30]. Light and electron microscopic observations in a rat SCI experiment demonstrated that hippocampal neurospheres injected into the SAS attached to the pial surface at the lesion and subsequently invaded extensively into the spinal cord tissue through the pia mater, although the pia mater over the lesion showed no obvious open damage [31].

Donor cells transplanted on the surface of the astrocyte scar tissue show a variety of migration modes

The geometry and composition of astrogliosis, including the astrocyte scar, depends on how and where it is formed (Box 3). At the root exit or entry zones of motor and sensory nerves, reactive astrocytes extend long processes following the contours of the nerve roots (the astrocyte outgrowth) (Box 3). In our study, donor cells appeared to enter the nerve autonomously and to use the astrocyte outgrowth as the main scaffold for migration [10]. On

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and within the astrocyte scar, a surprising variety of cell migration modes was observed, reminiscent of homotypic, glia- and neuron-guided behaviour of newborn neurons in the developing CNS [32] (Figure 2 A,B,C). Moreover, the cells were aligned in chains along elongated astrocyte scar processes expressing glial fibrillary acidic protein (GFAP), resembling the rostral migratory stream where neuroblasts migrate within a GFAP+, tube-like structure [33, 34] (Figure 2D). Preservation of the structure of the astrocyte scar thus seemed to aid rather than to inhibit integration of donor cells. It seems then that intraparenchymal injection risks destruction of critical structural and chemical cues needed for axon regeneration following cell transplantation. Indeed, intraparenchymal injection of donor cells into the auditory nerve failed to restore nerve function (Figure 1). In this experiment, the nerve was treated with chondroitinase ABC (ChABC) both at the time of nerve injury and at cell transplantation, which might facilitate interactions between the donor cells and the gliotic auditory nerve [10]. In the astrocyte scar, ChABC aids regeneration by digesting glycosaminoglycan (GAG) chains of CSPGs (chondroitin sulfate proteoglycans) that interact with receptors on growing neurons that prevent axon regeneration [35-37]. With ChABC, axon elongation is facilitated by such a receptor-mediated mechanism when donor cells express the CSPG side chain receptors [38, 39]. It is not known if the N33 cells used in our auditory nerve experiments express the CSPG side chain receptors but ChABC works through multiple mechanisms and can modify the immuno-inflammatory environment of the injured CNS to coax it to be pro-generative niche [40, 41]. Characteristics of CSPGs in the astrocyte scar in SCI are pro-regenerative or regeneration-inhibitory [42, 43] and hence one future direction could be to enhance the former and to remove the latter using yet-unidentified pharmacological agents to enhance its efficacy.

“The astrocyte outgrowth - Schwann cell complex” connects the CNS and PNS

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The anatomical relationships between astrocytes and Schwann cells and the basal lamina (BL) in the normal condition are maintained even in the astrocyte outgrowth [44-46] (Figure 3A-C) (Box 3). Intriguingly, ultrastructural analysis shows that the astrocyte outgrowth directly and extensively apposes with Schwann cells within common basal lamina tubes, providing a route for regeneration between the CNS and the PNS (peripheral nervous system) and decreasing the mutual repulsion that normally exists at this boundary [45] (Figure 3B). This “astrocyte outgrowth-Schwann cell complex”, or AO-SC complex, may be exploited for regeneration across the CNS-PNS boundary (Figure 3C). Under the administration of neurotrophins, blockade of the Nogo signaling pathway and application of ChABC to digest CSPG side chains allows regenerating sensory axons to pass the transitional zone (TZ, Box 3, Figure 3A) and reach the spinal cord [47]. It is unlikely that there is another route for regenerating sensory axons apart from the AO-SC complex within the common BL, as observed in motor neuron regeneration (see below).

Surface transplantation applied to the astrocyte outgrowth-Schwann cell complex as a potential treatment in motor neuron diseases and peripheral neuropathies

Given that the AO-SC complex provides a “bridge” between the CNS and peripheral targets (Figure 3C), we believe that motor neuron diseases and peripheral neuropathies, including ALS, **spinal muscular atrophy (SMA)**, **post-polio syndrome (PPS)** and **Guillain-Barré syndrome (GBS),** could be candidates for surface transplantation. Donor cells must traverse the BL to access the astrocyte outgrowth within it (Figure 3C). In our study on the auditory nerve, donor cells often entered the nerve in a homotypic fashion, interacting with each other in clusters at limited entry points (Figure 2A) [10]. Entry points probably arise through breaks in the BL that formed during nerve compression and subsequent degeneration (Figure 3C) (Figure 3E in ref. #10). Such discontinuities of the BL are not unusual in either normal or pathological tissue and

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the BL is traversed by numerous cell types including neural cells [48]. Conveniently, regenerating motor axons retrace their former pathways along the BL to form neuromuscular junctions (NMJ) on the same muscle fibers that they originally innervated [49]. One can speculate that appropriate motor neuron progenitors placed on the astrocyte outgrowth in the anterior nerve roots might extend regenerating axons along the AO-SC complex to form synapses with muscle fibers at the NMJ (Figure 3C). Motor neurons upregulate laminin receptors (integrins) in response to injury [50], so if donor cells with appropriate characteristics are selected, they might harness rich laminins within the BL for their axon growth (Box 3). In turn, the soma of transplanted cells on the surface of the astrocyte scar are expected to be innervated by increased projections from upper motor neurons as observed in SMA [51] (Figure 3C).

Reactive astrocytes for axon guidance in deeper CNS regions

Astrocyte scar tissue in NDDs with deeper CNS lesions differs from that in our auditory nerve injury model, not least because it does not include the AO-SC complex. We believe that surface transplantation could be clinically relevant in these NDDs as well, although the specific nature of astrogliosis must be taken into account for each condition (see Box 4). In an experiment where human neural stem cells (hNSCs) were injected into the striatum of a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced animal model of PD, local astrocytes were recruited to the lesion site where they formed a scaffold for chains of transplanted, migrating cells [52], similar to those observed in the auditory astrocyte scar model [10]. In human PD, astrogliosis is limited and a mature astrocyte scar usually does not develop [53] and hence reactive astrocytes may play key roles in donor cell migration as evidenced in the experiment cited above [52]. We believe that transplantation of donor cells to the surface of the insula could provide an alternative to the intraparenchymal route. The putamen, a portion of the striatum,

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which with the subthalamic nucleus is one of the main targets for PD treatment, lies 10 mm (probably less in the aged) below the insular surface [54], which can be reached through the Sylvian fissure. One crucial factor that determines the applicability of surface transplantation is the 'shallowness' - i.e., how far the lesion exists from the surface of host nervous tissue. As evidenced in the auditory astrocyte scar model, the shallower the scaffolds guiding to the final target from the surface, the easier it is for donor cells to reach the target. It is possible that this principle extrapolates to diseases in which lower motor neurons are compromised, including ALS, SMA, PPS and GBS (see above).

An essential requisite for donor cells to reach their targets within the CNS is a pro-regenerative, intraparenchymal path for migration. The hippocampus is one of the main potential targets for cell transplantation in AD. In one damaged hippocampus model, donor cells migrated away from the transplantation site and were distributed throughout the lesioned CA1 (Cornu ammonis 1) field, with some associated functional recovery [55], suggesting the existence of an appropriate intraparenchymal path for donor cell migration. This is supported by the observation that new progenitor cells generated in the subventricular zone (SVZ) following cortical injury migrated toward the lesion using reactive astrocytes as the scaffold [56]. In human AD, astrocyte scars such as those observed in SCI do not occur [57] and instead hypertrophic reactive astrocytes surround and wrap amyloid beta deposition (senile plaques) [58, 59]. Such sequestration of toxic aggregates from healthy parts of the brain may provide a favorable, pro-regenerative migration route [43]. Reactive astrocytes not only upregulate production of extracellular matrix (ECM) proteins and adhesion molecules, including growth-promoting molecules, laminin, N-cadherin, NCAM (neural cell adhesion molecule) and fibronectin [60-63] but also become a rich source of chemokines for axon elongation [64, 65]. Stromal cell-derived factor-1 (SDF-1, CXCL12) is expressed on reactive astrocytes and CXCL12 and its receptor CXCR4 plays crucial role in reactive astrocyte-assisted cell homing

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to lesioned areas [66, 67]. The surface of the hippocampal fissure (sulcus) would be a suitable site for testing cell delivery because it is very close to important anatomical structures in the hippocampus, including the dentate gyrus and CA1 and 3 (less than 1 mm, see Fig. 2A1 and B1 in [68]). Whilst this idea is speculative, we believe that the potential benefits justify exploratory experiments.

Concluding remarks and future perspectives

In this Opinion, we argue that astrogliosis, including the astrocyte scar, far from being a barrier, provides an important structural and biochemical scaffold for cell transplantation and regeneration. Studies in the auditory nerve model have shown that the astrocyte scar, if not physically disrupted, can be harnessed by surface delivery of appropriate donor cells for functional integration into the host (see Outstanding Questions). We speculate that deep-seated reactive astrocytes might similarly be harnessed by donor cells to treat a number of NDDs and that the potential benefits justify exploratory experiments in appropriate animal models This should not only enhance our understanding of astrogliosis and the astrocyte scar but also propel, we hope, the progress of cell-based therapies toward more effective clinical translation for nervous system repair.

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Box 1: The auditory astrocyte scar model

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In the auditory astrocyte scar model, mechanical injury was applied to the CNS portion of the auditory nerve (AuN) (large red arrow in Figure I) to induce astrocyte scar formation [10, 69-74]. In this model, neurons in the cochlear nuclei within the brainstem died trans-neuronally following death of auditory spiral ganglion neurons (small red arrows) [69, 73]. To simulate chronic aspects of NDDs, we waited 5 weeks after compression to establish an astrocyte scar before cell transplantation [10]. By comparison, in adult murine spinal cord injury, astrocyte scars form after two weeks [42]. We assessed the impact of compression on the host auditory nerve and nearby cochlear nucleus after 1, 4 and 25 weeks by immunofluorescence, immunoblotting and mRNA analysis for GFAP (glial fibrillary acidic protein) and CSPG (Neurocan) [10]. GFAP expression continued with sustained accumulation of CSPG (Neurocan) after compression, but nestin expression was only transient, providing strong evidence for the formation of a mature astrocyte scar.

Box 2: N33 cells

The donor cells used in our auditory nerve injury study [10, 71], US/VOT-N33, are conditionally immortal cells derived from the ventral otocyst (the inner ear anlage) of an ED10.5 Immortomouse© embryo and selected for markers of auditory spiral ganglion neurons [75, 76]. These cells are clonal and their preparation is consistent between experiments with no requirement for time-consuming, expensive and complex conditioning. Cell variability is much less than that of pluripotent stem cells, in which phenotypic stability is not entirely predictable [24, 77-79]. The N33 cells proliferate at 33°C in the presence of γ-interferon but differentiate both in vitro and in vivo to express both structural and molecular markers of auditory spiral ganglion neurons at 37-39°C in the absence of γ-interferon [71, 75, 76]. Further studies with immature cells such as tissue-specific stem cells or progenitor cells are warranted because they should provide insight into the selection of appropriate donor cells for NDDs.

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Box 3: Transitional zone, basal lamina, Schwann cells and astrocyte outgrowth

The transitional zone

Spinal and cranial nerves normally project into peripheral nerves at an interface called the transitional zone (TZ) (Figure II (A), Figure 3A) [44]. In the CNS, myelin sheaths are formed by oligodendrocytes and the supporting tissue is astrocytic whereas in the peripheral tissue, axons are enwrapped by Schwann cells in the endoneurium, although axons project through the TZ. The TZ can be visualized with antibodies to GFAP because this intermediate filament protein is expressed only by astrocytes in the CNS (Figure II (A), Figure 3A).

Basal lamina

The basal lamina (BL) adjacent to the astrocytes on the surface of the CNS (the glia limitans) and the TZ region is continuous with that investing Schwann cells in the peripheral nerve [45] (Figure 3A). The BL is an electron-dense layer of extracellular matrix (ECM) containing a sheet-like basement membrane of collagen type IV that acts as a scaffold to integrate proteins such as laminin and fibronectin [80]. For regeneration, the BLs provide a guide for cell migration and axon elongation [81] (Figure 3C).

Schwann cells

Following axon degeneration, Schwann cell columns (bands of Bungner) form within the basal lamina tubes that enclosed myelinating Schwann cells and their axons before nerve injury/degeneration. Schwann cell columns consist of myelin- and Remak-cell-derived repair Schwann cells in degenerated nerve [82, 83] (Figure 3B,C). Schwann cells rarely die after nerve injury because they have autocrine survival circuits [84] and provide a rich source of pro-

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regenerative molecules including glial cell-derived neurotrophic factor (GDNF), BDNF, neurotrophin-3 (NT-3), nerve growth factor (NGF) and cytokines that induce axon growth [83, 85, 86]. In the auditory astrocyte scar model, a marked inward migration of cells transplanted on the surface of astrogliotic auditory nerve has been observed [10], implying that the astrocyte scar contains attractants such as BDNF, most likely from hair cells and Schwann cells (Box 1).

The astrocyte outgrowth (the glial outgrowth)

At the root exit and entry zones, the processes of reactive astrocytes follow the contours of the nerve roots (Figure II (B)). It has been repeatedly described in lower motor neuron diseases [87, 88]. It was described for the first time as “glial bundles” in SMA [89], was later reported in ALS [90] and also linked to PPS in the anterior roots of a long-term polio survivor [91]. Ultrastructural studies revealed that the astrocyte outgrowth is composed of numerous astrocytic processes enclosed by the BL [90, 92] (Figure 3A,B).

Box 4. Surface transplantation, caveats

Cell transplantation involves complex interactions between donor cells, the host environment and changes in tissue pathology. Animal models cannot easily recreate human tissue pathology, especially those associated with longer periods of tissue degeneration and gliosis. This is further complicated by the wide range of potential donor cell types, cell delivery methods and assessments of functional recovery. Thus our ability to predict the outcome of cell transplantation experiments is difficult and both models and treatments for different conditions will almost certainly have to be closely tailored to each tissue and its pathology. The attraction of the auditory nerve model is that it allows relatively tight control of many of these variables (see Box 1) so that we can assess them in terms of functional recovery and more successfully translate the outcomes to realistic clinical pathologies of the auditory nerve. Nevertheless,

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whilst the model can indicate potentially important principles of cell transplantation there are important caveats to wider application to other forms of nerve injury and neurodegeneration. First, surface transplantation to the auditory nerve has not been tested in human patients and it is only through clinical trials that the true impact can be assessed. Second, the potential for donor cells to migrate greater distances to the target tissue is hard to predict and almost certainly dependent on the nature of tissue degeneration and on the selection of the donor cell type. This is particularly important for deeper lesions with more complex pathologies, including spinal cord injury. Third, the astrocytic response occurs whenever CNS neurons die but it differs between tissues and there can be progressive changes ranging from mild and reversible astrogliosis to the formation of more permanent astrocyte scars [43, 93-95]. Our auditory nerve model does not lead to the layered astrocytic scar formed with different cell-types [10] as observed in spinal cord injury [43]. Fourth, our auditory nerve model compared intra-neural injection with surface delivery in conjunction with application of Chondroitin ABC, the specific function of which requires further study. Despite these caveats, minimizing structural disruption to host tissue is a general principle that deserves further analysis in other pathologies and surface transplantation involves less invasive and possibly more measured entry of donor cells into host tissue. Note that it differs from the cell sheet technique, in which tissue lesions are covered with a layer of donor cells that do not individually penetrate the host [96].

Figure legends

Figure 1. **Surface transplantation vs. traditional intraparenchymal injection of donor cells to the auditory nerve**

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Views of the right cerebellopontine angle after removal of the suboccipital bone. (A) Donor cells placed on the surface of an astrogliotic auditory nerve (thick curved arrows) autonomously migrate (thin curved arrows), differentiate and form synapses with hair cells (HC) and with neurons in the cochlear nucleus (CN) [10]. IAM, internal auditory meatus. (B) With traditional intraparenchymal injection (straight arrow), donor cells show apoptotic cell death with little sign of survival, migration and differentiation [10]. AuN, auditory nerve; Cr, retracted cerebellar hemisphere.

Figure. 2. **Donor cell behaviour observed in the auditory astrocyte scar model.**

(A) A chain of donor cells (arrows) entered an astrogliotic auditory nerve from its surface (dotted lines) with homotypic interactions. (B) Glia-guided migration of transplanted cells. A transplanted cell (arrow) intimately related to GFAP+ process (arrowheads) derived from the astrocyte scar. (C) Neuron-guided migration of transplanted cells. GFP+ Tuj1+ cell (arrow) attached to residual neurons (arrowheads). (D) Transplanted cells (arrows) formed chains within GFAP+ sheaths (arrowheads). Scale bars: 20 μm in A, B upper; 8 μm in B lower; 25 μm in C; 10 μm in D. (Reproduced from ref. #10 with permission).

Figure 3. **The transitional zone, the astrocyte scar and the astrocyte outgrowth - Schwann cell complex** (A) The transitional zone is the interface between the central and peripheral portions of the nerve (see Box 3). The basal lamina (BL) investing Schwann cells is continuous with that of the glia limitans on the surface of astrocytes (see Box 3). (B) In neurodegeneration, the cellular strands of the repair Schwann cells (bands of Büngner) are formed (see Box 3 for detail). The strands are shown here in a simplified fashion but in fact they are markedly elongated, overlapped and often branched (see ref. # 82). Red dotted lines indicate possible regeneration tracks (see below).

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(C) Axons grow on pro-regenerative Schwann cell columns within laminin- and fibronectin-rich basal lamina tubes. The thin arrow indicates the tip of a tube introduced to the target region to transplant donor cells endoscopically. Asterisks indicate discontinuities of the BL. A neuromuscular junction is shown to the right. The thick grey arrow at the top left of the image indicates possible plastic innervation from sprouts and/or upper neural tracts running on the astrocyte scar. MF, muscle fiber; NT, nerve terminal.

Figure I. **The method to induce the astrocyte scar in the auditory system.** With compression injury applied to the CNS portion of the auditory nerve (AuN) (large red arrow), neurodegeneration proceeds both centrifugally and centripetally (small red arrows) with death of auditory spiral ganglion cells (SGC) and cochlear nucleus cells (CN), respectively. With CN cell death a prominent astrocyte outgrowth is induced (see Box 3). Auditory sensory hair cells produce BDNF/neurotrophin-3 (NT-3) and they do not degenerate. AuN, auditory nerve; fIAM, fundus of the internal auditory meatus (IAM); HC, hair cell; TZ, the transitional zone (see Box 3) (modified from ref. #10 with permission).

Figure II. **Normal shape of the transitional zone and the astrocyte outgrowth following auditory nerve compression**. (A) Normal shape of the transitional zone (down-pointing vertical arrows) of the auditory nerve (AuN). AuN-C, CNS portion of the auditory nerve; AuN-P, PNS portion of the auditory nerve. Oblique arrows indicate auditory spiral ganglion cells. (B) Mechanical compression applied to the AuN-C (double arrows) induced the astrocyte outgrowth (arrow heads). Asterisks indicate some of astrocyte scar processes projecting into small bony canals beyond the fundus of the internal auditory canal. Note thinning of the auditory nerve due to compression-induced nerve atrophy. Oblique arrows indicate residual

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auditory spiral ganglion cells after auditory nerve compression. Scale bars, 200 μm. Comp, compression. (modified from ref. #10 with permission)

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Glossary

Alzheimer's disease (AD): A neurodegenerative disease and a leading cause of dementia. One of the pathophysiological signatures of the disease is deposition of amyloid plaques and neurofibrillary tangles, which are typically observed earlier (relative to other brain regions) in the medial temporal lobe including the parahippocampal gyrus and the hippocampus.

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Amyotrophic lateral sclerosis (ALS): a neurodegenerative disease with progressive degeneration of the upper and lower motor neurons in the brain, brainstem and spinal cord that finally leads to paralyses in limb, bulbar and respiratory muscles.

Guillain-Barré syndrome (GBS): a peripheral neuropathy that is occasionally life-threatening. Prominent axon loss is observed in the axonal form of the disease, which is more aggressive than the demyelinating form.

Huntington's disease (HD): an autosomal dominant neurodegenerative disorder characterized, among other symptoms, by involuntary 'dancing' movements and psychiatric symptoms progressing to dementia. Neuronal loss is marked in the striatum and vast areas of the brain are compromised.

Multiple sclerosis (MS): the most frequent demyelinating disease of the CNS. It is characterized by demyelination, inflammation and axonal damage in the CNS due to unknown causes. At the demyelinated site, conduction impairment of nerve impulses occurs. The patients tend to repeat the remissions and aggravations of clinical manifestations.

Parkinson's disease (PD): a neurodegenerative disease caused mainly by degeneration of dopamine-containing neurons in the substantia nigra that project to the striatum (the caudate and putamen). Lewy bodies, an aggregation of α-synuclein, are observed in the lesions. Patients show slowness of movement, increased resistance to passive movements, shaking at rest, loss of postural reflexes, as well as a host of non-motor symptoms.

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Post-polio syndrome (PPS): a serious condition with newly manifesting weakness, pain and apnea after acute infection of poliomyelitis caused by poliovirus. Lower motor neurons degenerate but the pyramidal tract is typically spared. PPS is an urgent problem because many of the estimated 20 million polio survivors worldwide develop PPS.

Pyramidal tract: motor neural pathways are composed of two neurons, upper (UMNs) and lower motor neurons (LMNs). UMNs connecting the cerebral cortex and the brainstem or spinal cord form the pyramidal tracts (corticobulbar or corticospinal tract). LMNs connect the brainstem or spinal cord and effector organs such as skeletal muscles in the four extremities.

Spinal muscular atrophy: a genetic disease in children caused by a defect in the survival motor neurons (SMN) gene. It involves extensive degeneration of lower motor neurons in the spinal cord and of brain stem motor nuclei with the pyramidal tract preserved. In the most severe type, patients show flaccid quadriplegia and die earlier.

Temporal lobe epilepsy (TLE): a type of epilepsy that is often associated with pathologies in the hippocampal formation. In a substantial fraction of the patient population seizures are refractory to conventional antiepileptic drugs.

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Outstanding questions

Is intraparenchymal injection of donor cells the best way to ensure integration into the host neural circuitry with minimal damage to the host tissue? How does it compare with surface delivery?

Can astrogliosis and the structure of astrocyte scar tissue be harnessed for more effective integration of donor cells? What structural and soluble cues are most important for donor cell survival and growth?

In the absence of an immediate blood supply how can injected donor cells obtain nutrients from the host tissue to increase cell survival immediately after transplantation?

What is the optimum number of donor cells for cell transplantation to ensure minimal damage to the host with maximal donor cell survival and integration?

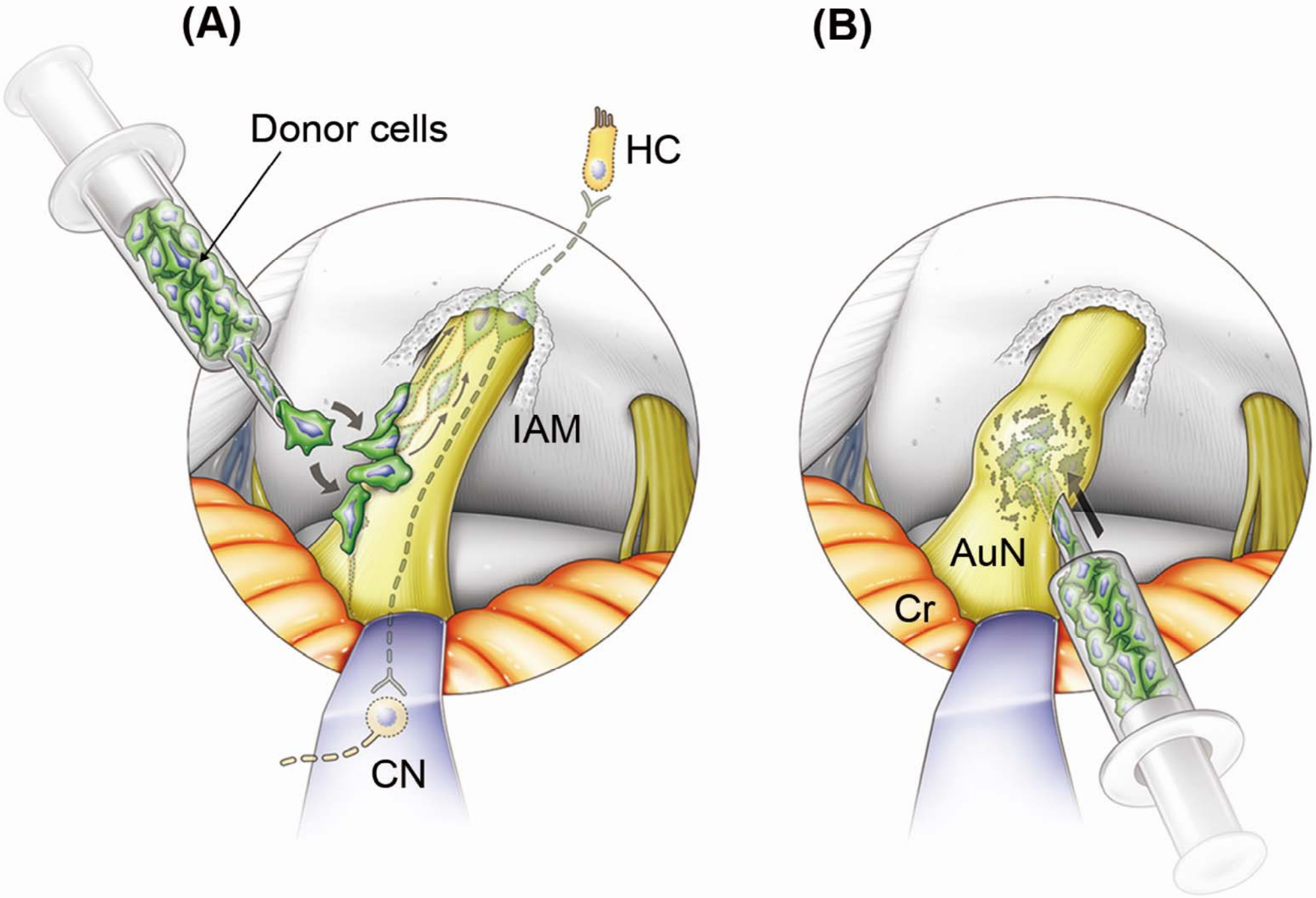
How can the growth of donor cells be facilitated either way across the boundaries between the central and peripheral nervous systems?

What biochemical cues guide the migration and growth of endogenous or exogenous cells toward damaged nervous tissue? Do they recapitulate the modes of cell migration observed during development?

How do the astrocytic response and the formation of the glial scar differ between different forms of nerve injury and neurodegenerative disorder?

Do the selection of donor cells and the method of cell delivery have to be matched specifically to the host tissue and pathology? How can different cell transplantation strategies be compared objectively to assess outcomes?

Figure 1



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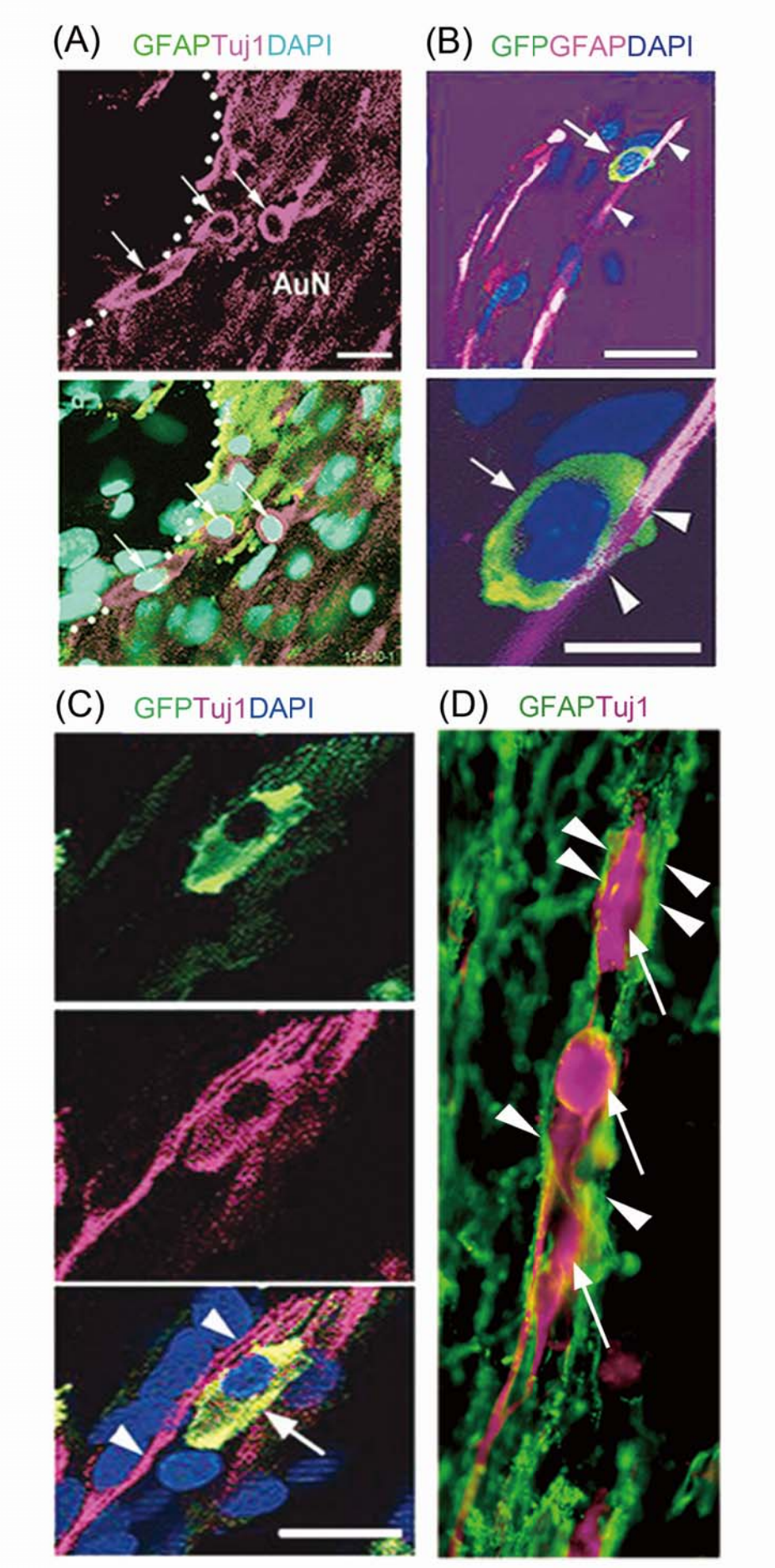

Figure 2

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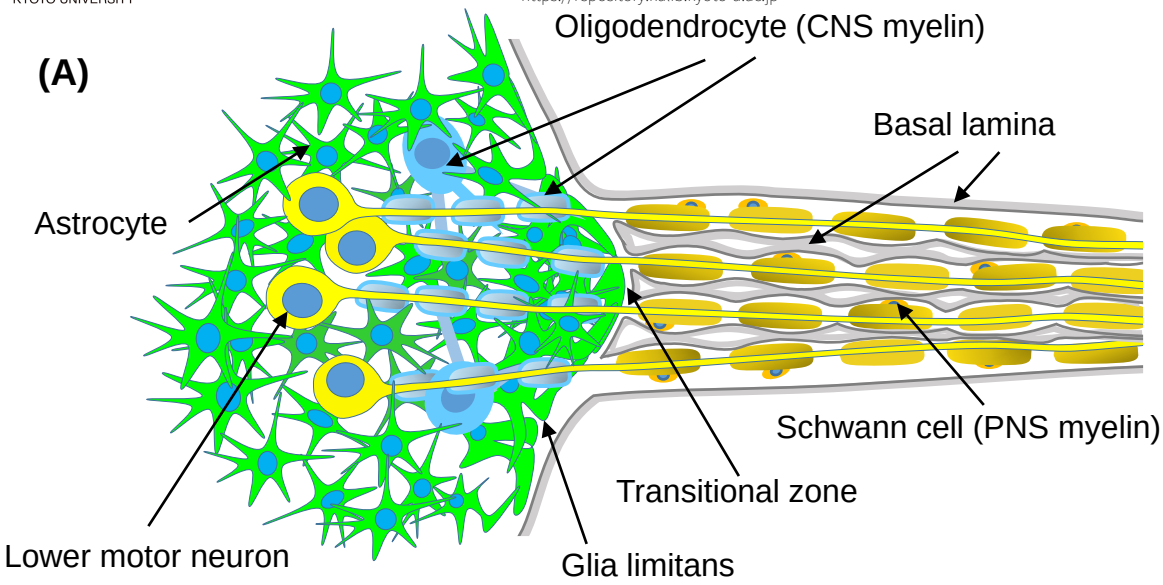
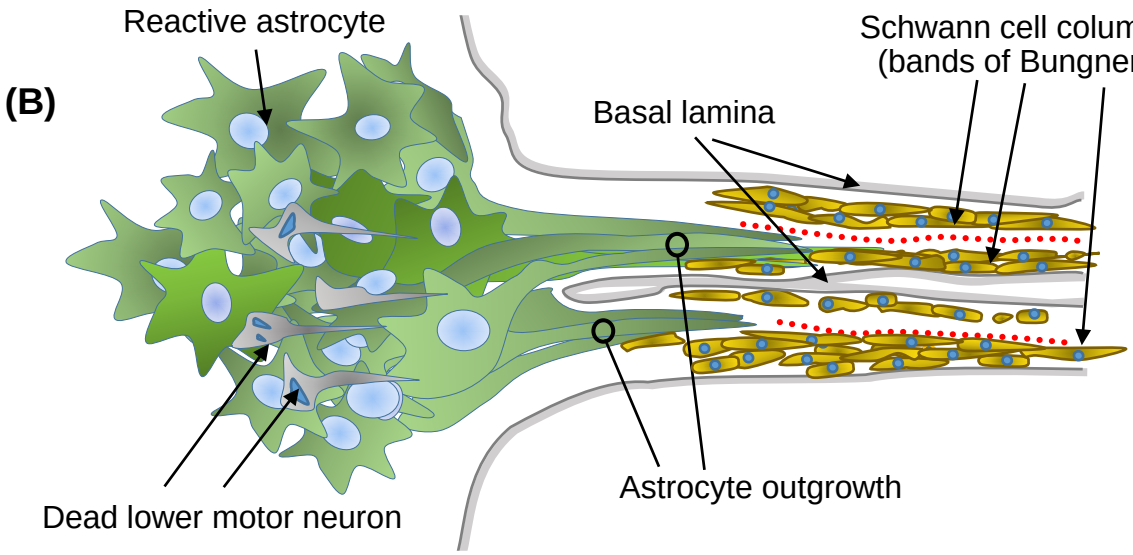




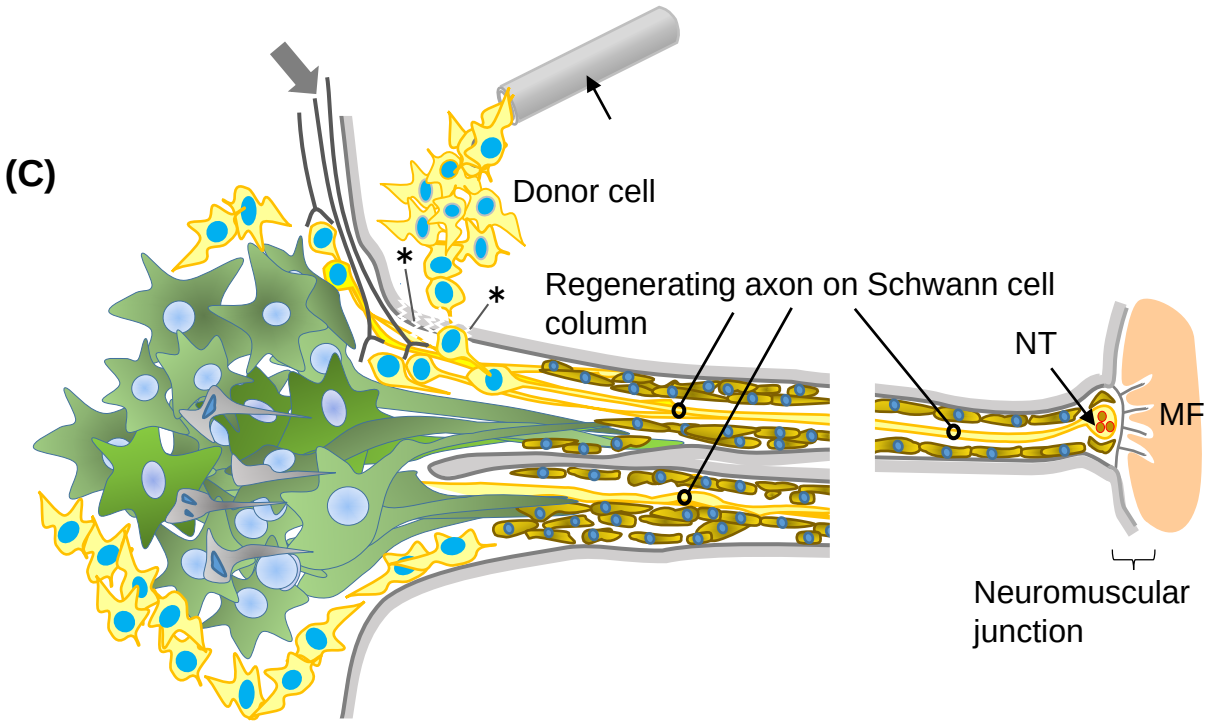
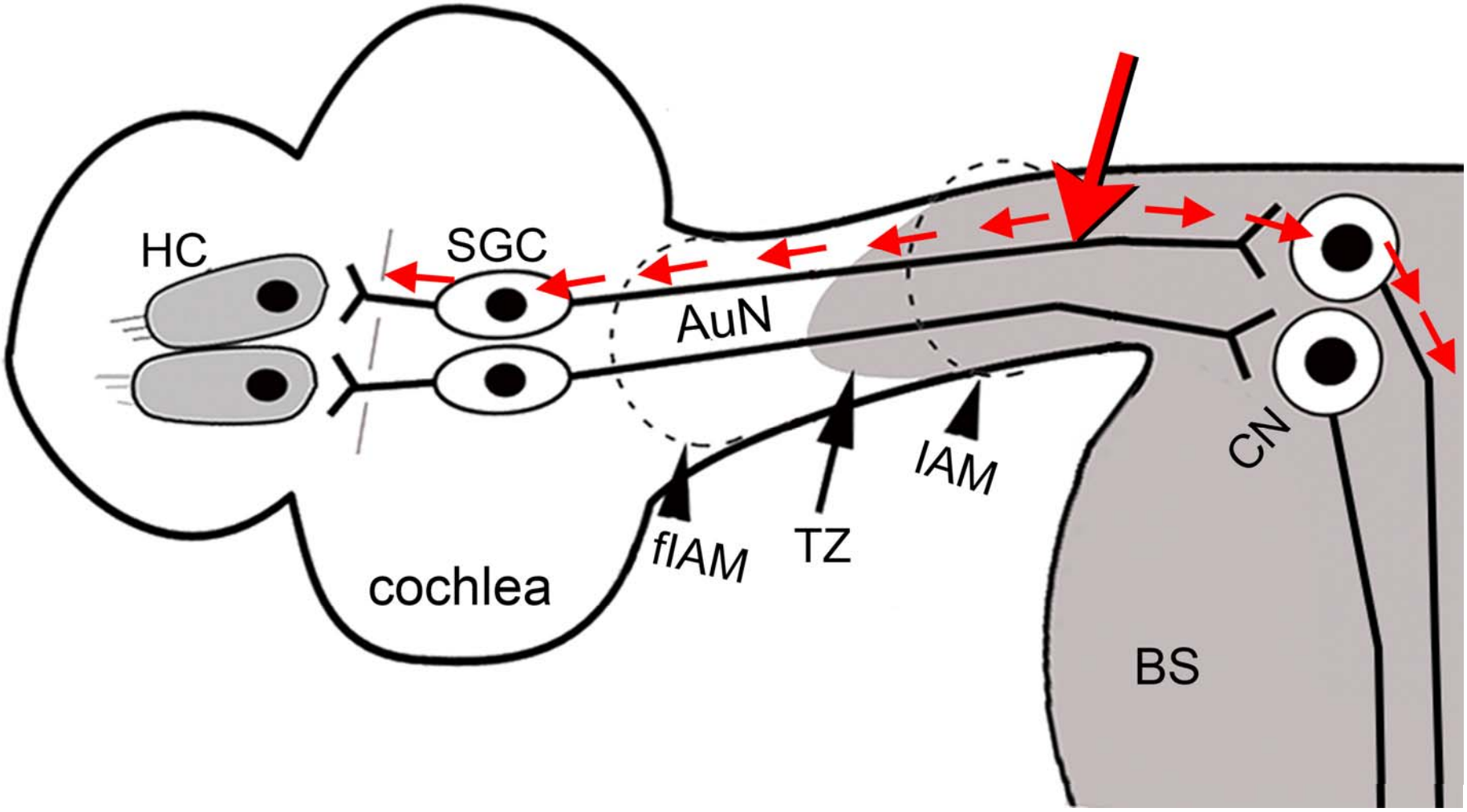
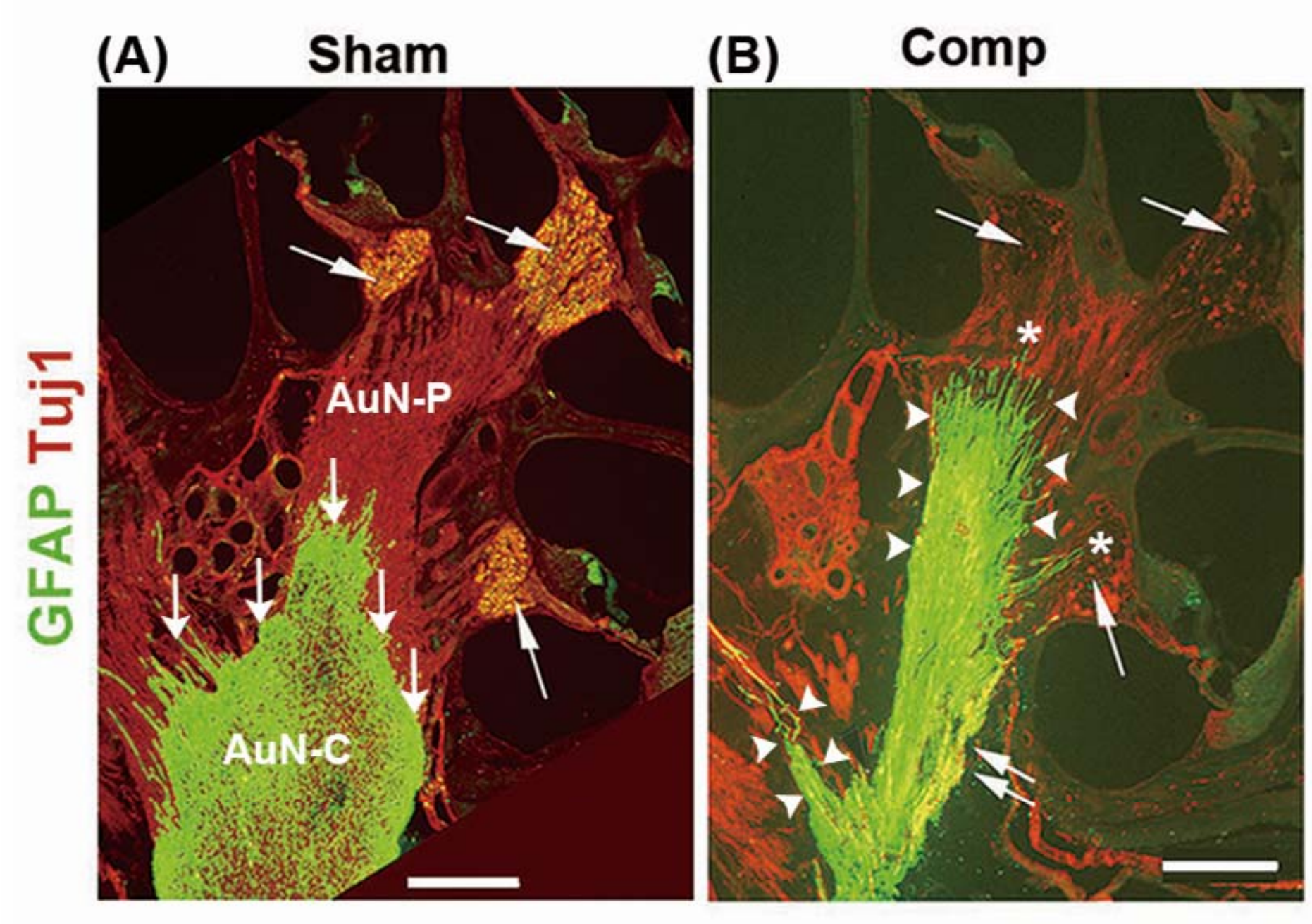


Figure 3



Box 1



Box 3